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Variation of fore wing shape in *Melipona mandacaia* Smith, 1863 (Hymenoptera, Meliponini) along its geographic range

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Abstract

Melipona mandacaia is a stingless bee species responsible for the pollination of many native plants in Brazil, South America. In spite of its ecological and economic importance, natural populations of *M. mandacaia* have been depleted because of deforestation. In order to evaluate the interpopulation morphometric structure of remaining populations, we carried out geometric morphometric studies based on fore wing shape in this native bee species. The grouping analysis by UPGMA revealed three distinct clusters and significant differences in fore wing size were observed ($p<0.001$) among populations. The three groups were also reflected in the first two principal components explaining about 60% of the total variation. These results indicate differentiation among populations, which can be regarded as unique management units. Therefore, efforts should be directed to the conservation of local populations of *M. mandacaia* to avoid the negative impacts of loss in pollination over plant species and environmental services.

Keywords
Bees, Apidae, principal component analysis (PCA), Meliponini, geometric morphometrics, Procrustes superimposition

Introduction

Stingless bees play a key role in natural and agricultural systems (Eardley et al. 2006) as they account for about 30% to 90% of pollination in native flora (Kerr 1997). Besides, these bees produce high-quality honey, propolis, pollen and wax used as food sources and/or as pharmaceutical products. However, human activities, such as deforestation and habitat loss, are a potential threat to these insects, leading to population declines (Fortuna and Bascompte 2006).

In Brazil, the stingless bees of the tribe Meliponini encompass most of native social bee taxa, in which *Melipona* is the species-richest genus (Michener 2007, Camargo and Pedro 2013). *Melipona mandacaia* Smith, 1863 is an endemic bee species from semiarid regions in northeastern Brazil, including one of the poorest Brazilian regions, named the "drought polygon". Being typical of the caatinga (dry bushland) biome, *M. mandacaia* is well adapted to high temperatures and reduced rainfall conditions (Carvalho et al. 2003, Batalha-Filho et al. 2011). The nests of this species are built in tree holes (Camara et al. 2004) and some populations have been raised in boxes by local farmers for honey production, representing a major portion of economy and food in traditional agriculture (Carvalho et al. 2003).

Because of its importance and vulnerability to deforestation, populations of stingless bees have been intensively studied using molecular (Francisco and Arias 2010, Tavares et al. 2013), behavioral (Kuhn-Neto et al. 2009, Palacio et al. 2010) and traditional morphometric methods (Nunes et al. 2007, Diniz-Filho and Bini 1994, Hepburn et al. 2005). Recently, the utilization of geometric morphometrics has increased in population (Tilde et al. 2000, Francoy et al. 2006, Nunes et al. 2008, Nunes et al. 2012), heritability (Monteiro et al. 2002), evolutionary (Bonatti et al. 2014) and reproductive (Carvalho et al. 2011) studies of bees. These reports have shown that geometric morphometric data are effective in the identification of groups and lineages (Francoy et al. 2008, Francoy et al. 2009, Bischoff et al. 2009).

In fact, morphometric analyses based on landmarks allow evaluating homologous morphological features that are independent of size (Francoy and Fonseca 2010). Furthermore, the geometric morphometrics of wings have been useful to establish the geographic origin of individuals of a single species (Bischoff et al. 2009, Nunes et al. 2012).

In spite of being a precise and simple technique to assess population diversity (Adams et al. 2004), no geometric morphometrics reports are available in *M. mandacaia.* Therefore, we focused on analyzing morphogenetic traits in populations of *M. mandacaia* in order to examine how populations are structured throughout their range.

Material and methods

The samples were collected in 15 localities (3–26 colonies per collection site) along the semiarid regions of the state of Bahia, northeastern Brazil. Right after collection, the specimens were stored in absolute ethanol for further morphometric analyses. The right fore wings were removed from 9 to 13 adult workers per colony, totaling 127 colonies and 1199 specimens (Table 1). Afterwards, they were placed onto glass slides and photographed using a stereomicroscope equipped with an image capture system (Leica Application Suite version 3.4.1).

Based on high-resolution images, two dimensional landmarks were digitized with the software TPSDIG2 (Rohlf 2006). A total of 12 landmarks were recorded in each fore wing for morphometric analyses (Figure 1).

Based on Cartesian coordinates, Procrustes superimposition and Principal Component Analysis (PCA) were performed using the software MORPHOJ version 2.0 (Klingenberg 2011) based on mean values of colonies. Subsequently, a cluster analysis using UPGMA (Unweighted Pair-Group Method with Arithmetic Average) was carried out with the software Past (Paleontological Statistics) version 1.81 (Hamer 2008). The cophenetic correlation coefficient was also calculated using Past.

A variance analysis (ANOVA) followed by a Tukey test was performed to evaluate centroid size. Shape and size of fore wings from each population were compared to altitude and geographic distance of colonies via a Mantel test in the software NTSYS version 2.02 (Rohlf 1993).

Table 1. Sampling sites of *Melipona mandacaia* with altitude, geographic coordinates and number of samples colonies and specimens.

Locality	Altitude (m)	Latitude (S)	Longitude (W)	Number of colonies	Number of specimens
Casa Nova	397	9°59'56.2"	42°28'50.5	10	100
Hidrolândia	492	11°15'45.4	42°07'26.1	10	100
Itaguaçu	44	11°00' 42”	42°23' 58”	6	60
Juazeiro	368	9°24'42"	40°29'55"	26	168
Macururé	357	9°10'03"	39°03'27"	7	82
Morpará	405	11°33'31"	43°16'51"	6	66
Morro do Chapéu	609	13°12'0"	40°19'18.1"	10	100
Muquem de São Francisco	560	12° 11' 55''	43° 49' 58''	4	41
Ourolândia	560	10°58'13"	41°04'59"	3	31
Paulo Afonso	243	9°24' 28”	38°13' 19”	5	50
Pilão Arcado	394	9°53'57.6	42°29'11.1"	7	68
Remanso	388	9°12'44.1"	42°01'29.2"	11	111
São Gabriel	692	11°13'44"	41°54'43"	15	150
Serra do Ramalho	438	13°33'45"	43°35'48"	3	32
Uibaí	582	11°20'13"	42°07'58"	4	40
TOTAL				127	1199

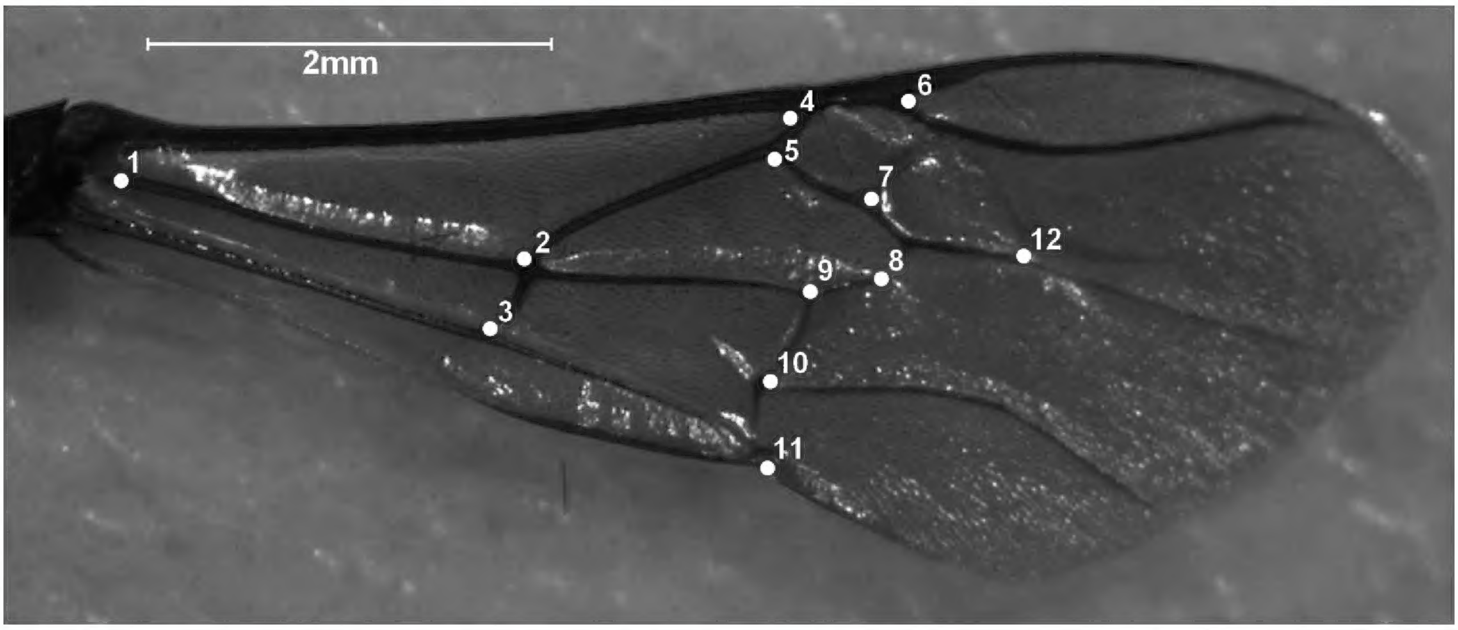

Figure 1. Right fore wing of *Melipona mandacaia* with 12 landmarks.

Results

The first two principal components explained about 60% of the variation in fore wing shape. Three groups were distinguished according to collection site in PCA, as follows: 1 – Pilão Arcado, Remanso and Casa Nova; 2 – Juazeiro, Macururé and Paulo Afonso; and 3 – Ourolândia, Morro do Chapéu, Serra do Ramalho, São Gabriel, Morpará, Itaguaçu da Bahia, Muquém de São Francisco, Uibaí and Hidrolândia (Figure 2).

Similarly, the dendrogram based on UPGMA (Figure 3) revealed three distinct clusters. It should be pointed out that the third group is composed of colonies from both sides of the São Francisco river (Serra do Ramalho and Muquém de São Francisco are located on the left margin while Morro do Chapéu, São Gabriel, Morpará, Itaguaçu da Bahia, Ourolândia, Uibaí and Hidrolândia are located on right margin of São Francisco River) with a cophenetic correlation coefficient of 84% (Figure 4).

Fore wing size was significantly different among colonies from distinct collection sites ($p<0.001$) (Figure 5) by the Tukey test. However, the fore wing shape was more informative in discriminating the groups than size, thus demonstrating the efficiency of geometric morphometrics.

Table 2. Comparison between matrixes of geographic distances, altitude and shape and size of fore wings using Mantel's test with 5000 permutations.

Variables	R	p
Geographic distance × forewing shape	0.349	0.0122*
Geographic distance × forewing size	0.438	0.0008**
Altitude × forewing size	-0.149	0.8420ns
Altitude × forewing shape	-0.19	0.9236ns
forewing size × forewing shape	0.884	0.0002**

ns ($p>0.05$); * ($p<0.05$); ** ($p<0.001$)

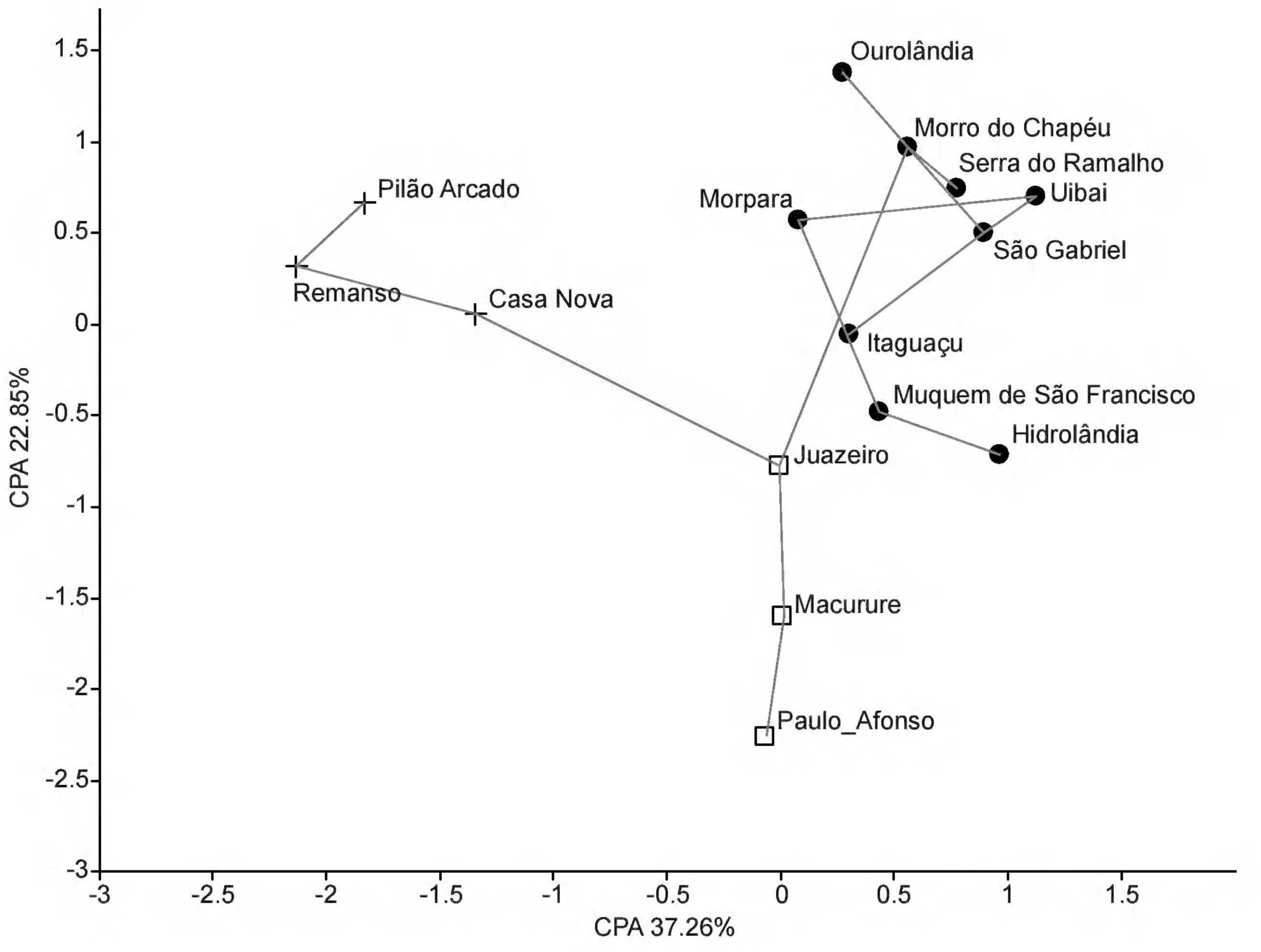

Figure 2. Analysis of Principal Components and Minimum Spanning Tree of fore wings of Melipona mandacaia.

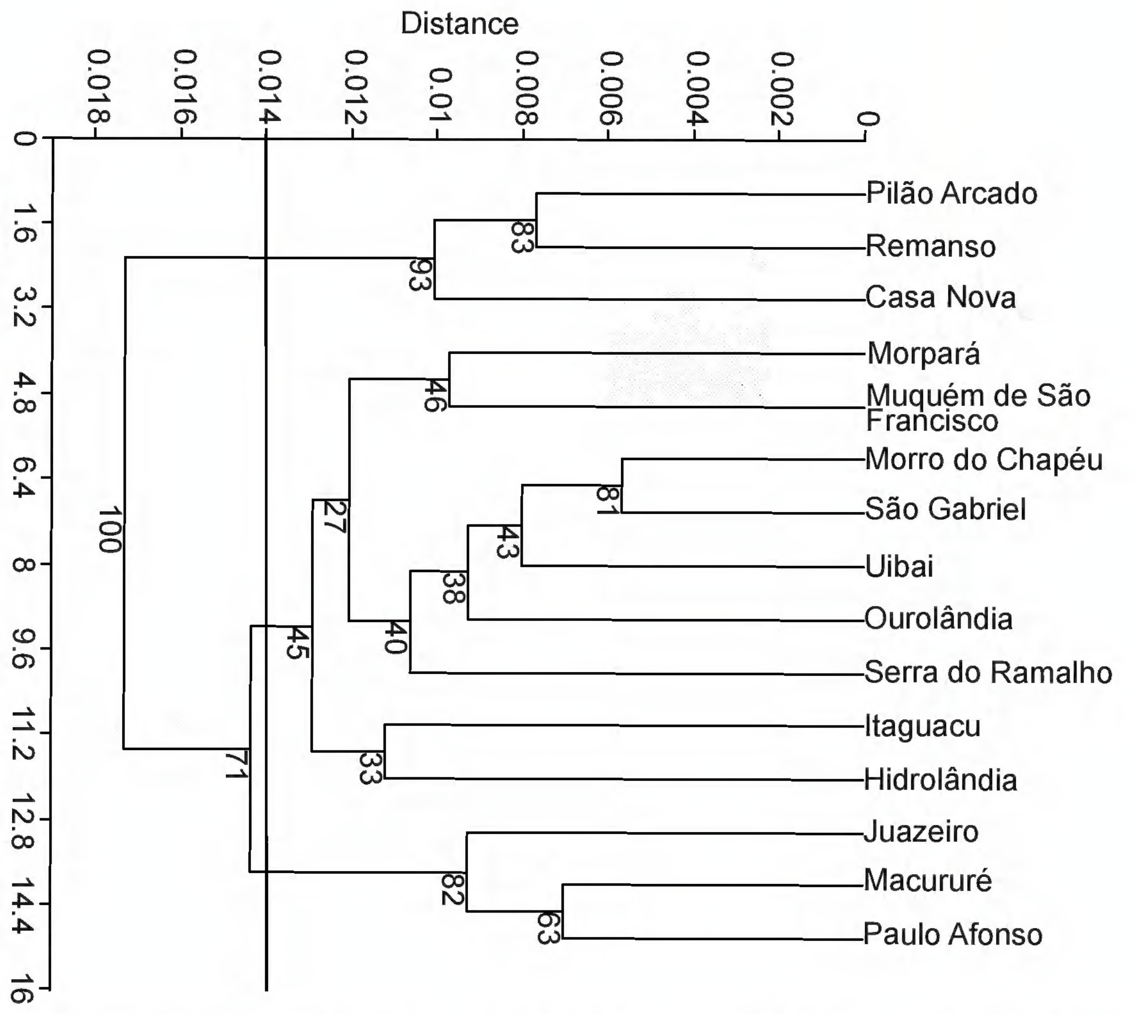

Figure 3. UPGMA dendrogram of morphological relatedness of fore wings based on Euclidean distance among colonies of Melipona mandacaia with bootstrap values (after 10,000 repetitions).

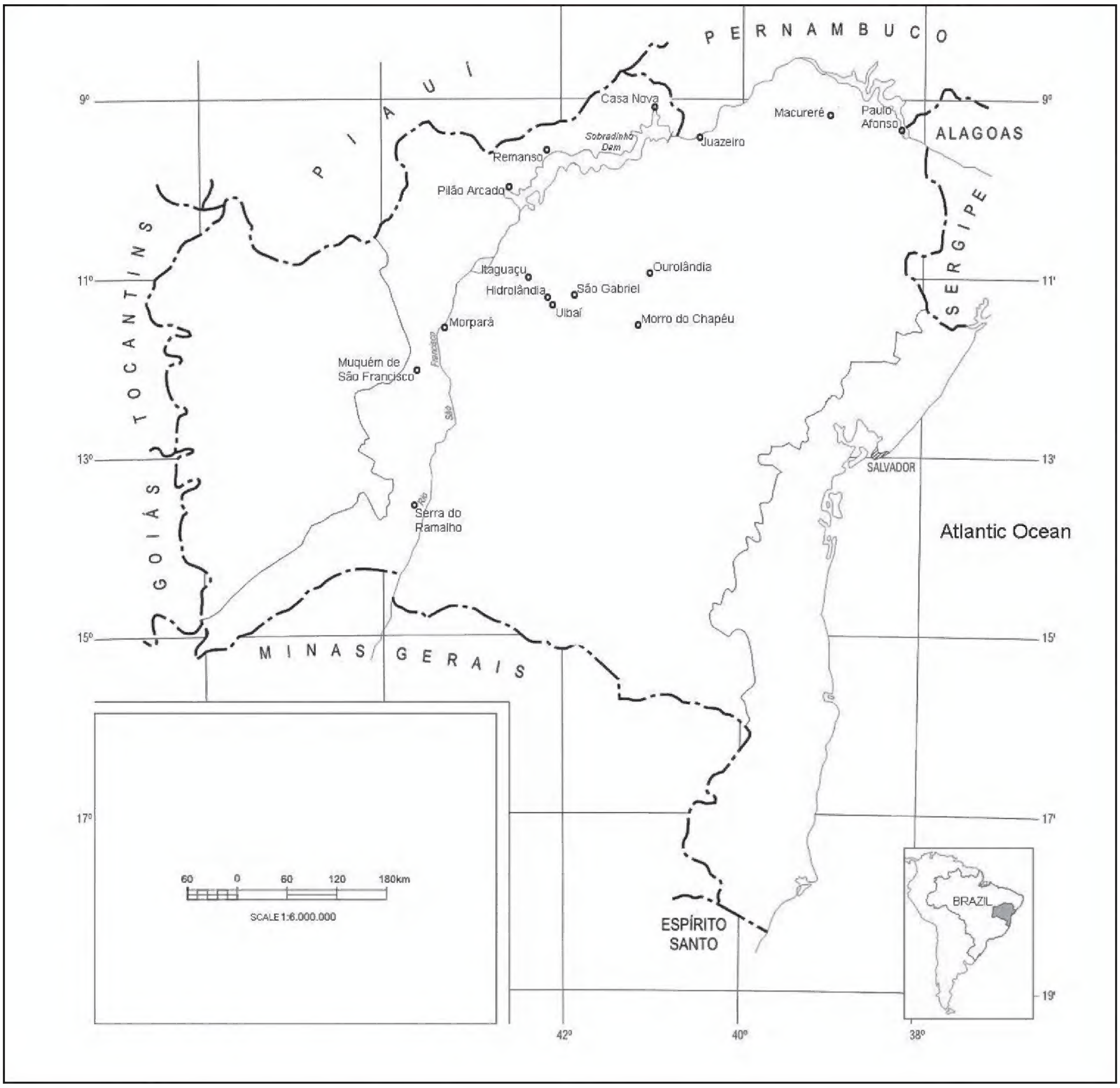

Figure 4. Map of Bahia, in Brazil, with sampling sites of *Melipona mandacaia*.

The Mantel test revealed no correlation between both size x altitude and shape x altitude ($p>0.05$) (Table 2). On the other hand, both shape ($p<0.05$) and size ($p<0.001$) were weakly but significantly correlated to geographic distance in *M. mandacaia*.

Discussion

The present analyses indicated gene flow among individuals from the three morphogenetic groups of *M. mandacaia* (Figure 2) inasmuch as populations are weakly structured. This result suggests that São Francisco River should not act as an effective geographic barrier to the dispersal of individuals from both river margins.

Similarly, molecular markers revealed a moderate genetic structuring ($\Phi ST = 0.2961$) among these populations (Miranda et al. 2012). Only the Sobradinho

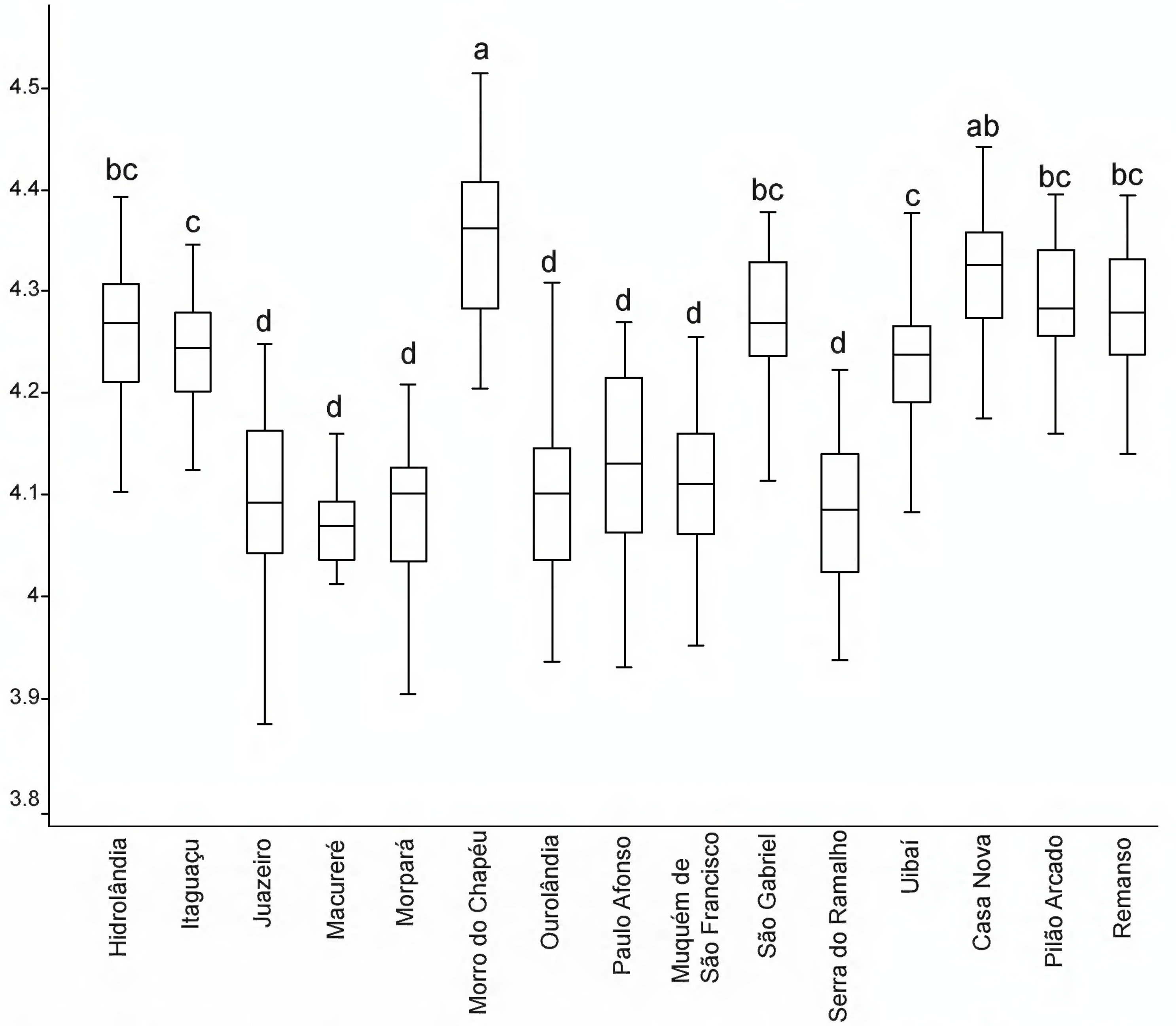

Figure 5. Boxplot of fore wing centroid size of *Melipona mandacaia* and comparison of mean values by a Tukey test (same letters represent no significant statistical differences).

reservoir (about 320 km of extension and a water surface of 4.214 km²) seems to disrupt gene flow between groups 1 and 2, as identified in this study (Figure 4). Thus, the construction of dams related to human disturbance seems to be responsible for the population structure associated with decreased genetic variation and high inbreeding in colonies of *M. mandacaia*. A similar result was reported in *Apis mellifera* L. (Miguel et al. 2007) and rodents (*Calomys expulsus* Lund, 1841) (Nascimento et al. 2011). Additional support to this hypothesis is that the Sobradinho dam was built about 40 years ago. This relatively long period is compatible with, at least, 10 generations of queen bees that could potentially lead to both fixation and losses of alleles (i.e. reduced genetic variation).

Moreover, previous reports have shown that fore wing size and shape of some *Melipona* species are influenced by both geographic distance and altitude, like that observed in *M. scutellaris* Latreille (Nunes et al. 2007), *M. quadrifasciata anthidioides* Lepeletier (Nunes et al. 2013) and *M. subnitida* Duke (Lima et al. 2014). Nonetheless,

no significant morphometric differences were observed in *M. mandacaia* in relation to altitude ($p>0.05$), but both fore wing size and shape were correlated to geographic distance ($p<0.001$ and $p<0.05$, respectively). This isolation-by-distance model in *M. mandacaia* can be explained by the eusocial behavior of these bees and their short flight range (about 2,000 m) (Araújo et al. 2004), causing restricted gene flow among populations.

Therefore, the morphometric analysis based on fore wingsis useful to evaluate the interpopulation genetic divergence of *M. mandacaia*, once they seem to be less susceptible to environmental influence. In this sense, studies of geometric morphometrics are useful to the identification of variation in populations and species of bees (Oleksa and Tofilski 2015) that are essential to conservation plans.

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References

Adams DC, Rohlf FJ, Slice DE (2004) Geometric morphometrics: ten years of progress following the 'revolution'. Italian Journal of Zoology 71: 5–16. doi: 10.1080/11250000409356545

Araújo ED, Costa M, Chaud-Netto J, Fowler HG (2004) Body size and flight distance in stingless bees (Hymenoptera: Meliponini): inference of flight range and possible ecological implications. Brazilian Journal of Biology 64(3B): 563–568. doi: 10.1590/S1519-69842004000400003

Batalha-Filho H, Waldschmidt AM, Alves RMO (2011) Distribuição potencial da abelha sem ferrão endêmica da caatinga, *Melipona mandacaia* (Hymenoptera, Apidae). Magistra, 23(3): 129–133.

Bischoff I, Schroeder S, Misof B (2009) Differentiation and range expansion of North American squash bees *Peponapis pruinosa* (Apidae: Apiformes) populations assessed by geometric wing morphometry. Annals of the Entomological Society of America 102: 60–69. doi: 10.1603/008.102.0106

Bonatti V, Simões ZLP, Franco FF, Francoy TM (2014) Evidence of at least two evolutionary lineages in *Melipona subnitida* (Apidae, Meliponini) suggested by mtDNA variability and geometric morphometrics of forewings. Naturwiss. doi: 10.1007/s00114-013-1123-5

Camara JQ, Sousa AH, Vasconcelos WE, Freitas RS, Maia PHS, Almeida JC, Maracajá PB. (2004) Estudos de meliponíneos, com ênfase *Melipona subnitida* no município de Jandaíra, RN. Revista de biologia e ciências da terra 4(1): 1–20. http://www.abelhanativa.com.br/artigos/meliponideosmjandaira.pdf

Camargo JMF, Pedro SEM (2013) Meliponini Lepeletier, 1836. In: Moure JS, Urban D, Melo GAR (Eds) Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region. http://www.moure.cria.org.br/catalogue [accessed Dec/20/2013]

Carvalho CAL, Alves RMO, Souza BA (2003) Criação de abelhas sem ferrão: aspectos práticos. Universidade Federal da Bahia, Cruz das Almas.

Carvalho CAL, Santos WS, Nunes LA, Souza BA, Zilse GAC, Alves RMO (2011) Offspring analysis in a polygyne colony of *Melipona scutellaris* (Hymenoptera: Apidae) by means of morphometric analyses. Sociobiology 57(2): 347–354.

Diniz-Filho JAF, Bini LM (1994) Space-free correlation between morphometric and climatic data: a multivariate analysis of Africanized honey bees (*Apis mellifera* L.). Brazilian Global Ecolology and Biogeography 4: 195–202. doi: 10.2307/2997651

Eardley C, Roth D, Clarke J, Buchmann S, Gemmil B (2006) Pollinators and pollination: a resource book for policy and practice. African Pollinators, Pretoria, South African.

Fortuna MA, Bascompte J (2006) Habitat loss and the structure of plant–animal mutualistic networks. Ecology Letters 9: 281–286. doi: 10.1111/j.1461-0248.2005.00868.x

Francisco FO, Arias MA (2010) Inferences of evolutionary and ecological events that influenced the population structure of *Plebeia remota*, a stingless bee from Brazil. Apidologie 41: 216–224. doi: 10.1051/apido/2009079

Francoy TM, Prado PRR, Gonçalves LS, Costa LF, Jong DD (2006) Morphometric diferences in a single wing cell can discriminate *Apis mellifera* racial types. Apidologie 37: 91–97. doi: 10.1051/apido:2005062

Francoy TM, Wittmann D, Drauschke M, Müller S, Steinhage V, Bezerra-Laure MAF, de Jong D, Gonçalves LS (2008) Identification of Africanized honey bees through wing morphometrics: two fast and efficient procedures. Apidologie 39: 488–494. doi: 10.1051/apido:2008028

Francoy TM, Silva RAO, Nunes-Silva P, Menezes C, Imperatriz-Fonseca VL (2009) Gender identification in five genera of stingless bees (Apidae, Meliponini) based on wing morphology. Genetics and Molecular Research 8: 207–214.

Francoy TM, Fonseca VLI (2010) A Morfometria Geométrica de asas e a identificação automática de espécies de abelhas. Oecologia Australis 14(1): 317–321. doi: 10.4257/oeco.2010.1401.20

Hamer Øy, Harper DAT (2008) PAST – Paleontological Estatistics, version 1.81. http://folk.uio.no/ohammer/past/ [accessed on 23/Jan/2012]

Hepburn HR, Radloff SE, Otis GW, Fuchs S, Verma LR, Ken T, Chaiyawong T, Tahmasebi G, Ebadi R, Wongsiri S (2005) *Apis florea*: morphometrics, classification and biogeography. Apidologie, 36: 359–376. doi: 10.1051/apido:2005023

Kerr WE (1997) Meliponicultura – A importância da meliponicultura para o País. Biotecnologia Ciência & Desenvolvimento 1(3): 42–44.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11: 353–357.doi: 10.1111/j.1755-0998.2010.02924.x

Kuhn-Neto B, Contrera FAL, Castro MS, Nieh JC (2009) Long distance foraging and recruitment by a stingless bee, *Melipona mandacaia*. Apidologie 40: 472–480. doi: 10.1051/apido/2009007

Lima CBS, Nunes LA, Ribeiro MF, Carvalho CAL (2014) Population structure of *Melipona subnitida* Ducke (Hymenoptera: Apidae: Meliponini) at the Southern limit of its distribution based on geometric morphometrics of forewings. Sociobiology 61(4): 478–482. doi: 10.13102/sociobiology.v61i4.478-482

Michener CD (2007) The Bees of the World. The John Hopkins University Press, Baltimore.

Miguel I, Liriondo M, Garnery L, Sheppard WS, Estonba A (2007) Gene flow within the M evolutionary lineage of *Apis mellifera*: role of the Pyrenees, isolation by distance and post-glacial re-colonization routes in the western Europe. Apidologie 38: 141–155. doi: 10.1051/apido:2007007

Miranda EA, Batalha-Filho H, Oliveira PS, Alves RMO, Campos LAO, Waldschmidt AM (2012) Genetic diversity of *Melipona mandacaia* Smith 1863 (Hymenoptera, Apidae), an endemic bee species from Brazilian caatinga, using ISSR. Psyche. doi: 10.1155/2012/372138

Monteiro LR, Diniz-Filho JAF, dos Reis SF, Araújo ED (2002) Geometric estimates of heritability in biological shape. Evolution. doi: 10.1554/0014-3820(2002)056[0563:GEOHIB]2.0.CO;2

Nascimento FF, Pereira LG, Geise L, Bezerra AMR, D'Andrea OS, Bonvicino CR (2011) Colonization process of the Brazilian common vesper mouse *Calomys expulsus* (Cricetidae, Sigmodontinae): A Biogeographic Hypothesis. Journal of Heredity 102(3): 260–268. doi: 10.1093/jhered/esr012

Nunes LA, Costa-Pinto MFF, Carneiro PLS, Pereira DG, Waldschmidt AM (2007) Divergência genética em *Melipona scutellaris* Latreille (Hymenoptera: Apidae) com base em caracteres morfológicos. Bioscience Journal 23: 1–9.

Nunes LA, Araujo ED, Carvalho CAL, Waldschmidt AM (2008) Population divergence of *Melipona quadrifasciata anthidioides* (Hymenoptera: Apidae) endemic to the semi-arid region of State of Bahia, Brazil. Sociobiology 52: 81–93.

Nunes LA, Araujo ED, Marchini LC, Moreti ACCC (2012) Variation morphogeometrics of Africanized honey bees (*Apis mellifera*) in Brazil. Iheringia. Série Zoologia. doi: 10.1590/S0073-47212012005000002

Nunes LA, Passos GB, Carvalho CAL, Araújo ED (2013) Size and shape in *Melipona quadrifasciata anthidioides* Lepeletier, 1836 (Hymenoptera; Meliponini). Brazilian Journal of Biology 73(4): 887–893.doi: 10.1590/S1519-69842013000400027

Oleksa A, Tofilski A (2015) Wing geometric morphometrics and microsatellite analysis provide similar discrimination of honey bee subspecies. Apidologie 46: 49–60. doi: 10.1007/s13592-014-0300-7

Palacio MA, Rodriguez E, Goncalves L, Bedascarrasbure E, Spivak M (2010) Hygienic behaviors of honey bees in response to broodexperimentally pin-killed or infected with *Ascosphaera apis*. Apidologie 41: 602–612. doi: 10.1051/apido/2010022

Rohlf FJ (2006) TpsDig2, digitize landmarks and outlines, version 2.10. Dept. Ecol. & Evol., St. Univ.New York, Stony Brook, New York.

Rohlf FJ, Marcus LF (1993) A revolution in morphometrics. Trends in Ecology and Evolution, Amsterdam.

Tavares MG, Pietrani NT, Durvale MC, Resende HC, Campos LAO (2013) Genetic divergence between *Melipona quadrifasciata* Lepeletier (Hymenoptera, Apidae) populations. Genetic Molecular Biology 36(1): 111–117. doi: 10.1590/S1415-47572013000100016

Tilde MAC, Fuchs S, Koeniger N, Cervancia CR (2000) Morphometric diversity of *Apis cerana* Fabr. within the Philippines. Apidologie 31: 249–263. doi: 10.1051/apido:2000120